CORRESPONDENCE

Assessment of Minimal Residual Disease in Standard-Risk AML

TO THE EDITOR: Ivey and colleagues (Feb. 4 issue)[1] tested the prognostic value of the presence of a mutation in the gene encoding nucleophosmin (*NPM1*) in patients with acute myeloid leukemia (AML) who were in complete hematologic remission after a second cycle of chemotherapy,[2] although the recovery of cell counts in peripheral blood was not required for a determination of such remission. Some of the patients were in remission after the first cycle of chemotherapy and some were not. Moreover, some of the patients in remission had normal blood counts and some did not. Other studies have shown the prognostic importance of obtaining normal blood counts over shorter durations of time and with fewer cycles required to achieve remission. Thus, we wonder whether the patients who did not have minimal residual disease were more likely to have a remission with normal cell counts and more likely to be in remission after the first cycle of chemotherapy than were patients who were found to have minimal residual disease.

Michele Baccarani, M.D.

University of Bologna
Bologna, Italy
michele.baccarani@unibo.it

No potential conflict of interest relevant to this letter was reported.

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2. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol 2003;21:4642-9.

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TO THE EDITOR: Ivey et al. describe the persistence of mutations in genes other than *NPM1* in the blood and marrow of patients with AML who were in complete hematologic remission. The authors also state that patients with persistent *NPM1* transcripts (the presence of which defined minimal residual disease) had a worse prognosis than those who did not carry this marker and that virtually all patients who had a relapse carried the *NPM1* mutation. At our center, in 32 consecutive patients (median age, 55 years) with cytogenetically normal AML carrying a de novo mutation in *NPM1* without internal tandem duplications in the gene encoding Fms-like tyrosine kinase 3 (*FLT3*-ITD) who had a complete remission after intensive chemotherapy, 14 (44%) had a relapse (all with the same *NPM1* mutation as the one observed at diagnosis), 12 (38%) remained in complete remission, and 6 (19%) (all of whom were >55 years of age) had disease that developed into either the myelodysplastic syndrome (in 5 patients) or primary myelofibrosis (in 1 patient) (Fig. 1A). In each of these 6 patients, a preleukemic clone with at least one mutation in *TET2*, *JAK2*, *ASXL1*, *IDH2*, or a gene encoding a spliceosome protein was found at the time of the AML diagnosis and was present after the development of either the myelodysplastic syndrome or primary myelofibrosis (Fig. 1B). However, the *NPM1* mutation was absent. Thus, although we agree that relapse does not arise from preleukemic clones of cells with wild-type *NPM1*, such clones may promote the development of the myelodysplastic syndrome or primary myelofibrosis, especially in older patients. Did Ivey et al. obtain data that support this conclusion and, if so, did they observe an age-specific association?

Sarah Morin-Zorman, M.D., Ph.D.

Hôpital Saint Louis
Paris, France
sarah.morin@aphp.fr

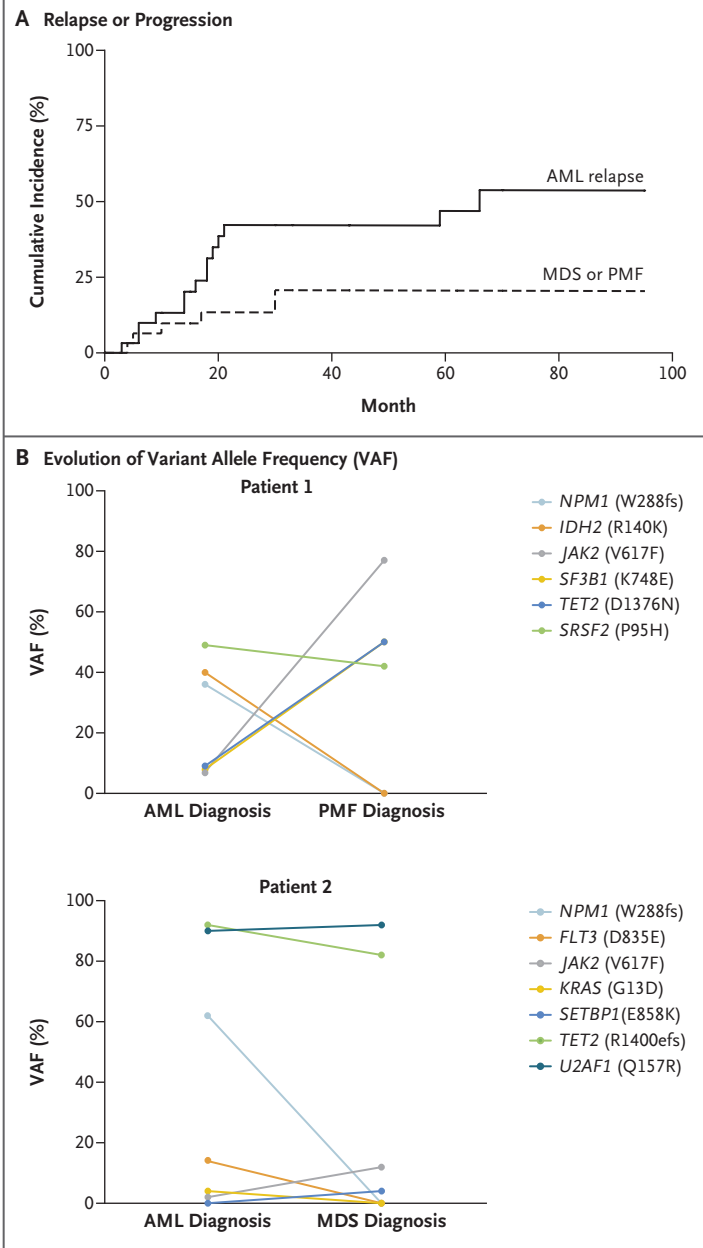

Figure 1. Relapse or Progression of Acute Myeloid Leukemia (AML) and Evolution of the Variant Allele Frequency in Mutated Genes.

Panel A shows the cumulative incidence of relapse and evolution to the myelodysplastic syndrome (MDS) or primary myelofibrosis (PMF) in 32 patients with cytogenetically normal AML with the *NPM1* mutation without internal tandem duplications in the gene encoding Fms-like tyrosine kinase 3 (*FLT3*-ITD) during the first complete remission. (The presence of the *NPM1* mutation without the *FLT3*-ITD genotype is associated with a better prognosis.) Panel B shows the evolution of the variant allele frequency in mutated genes in 2 patients at the time of AML diagnosis and at the time of the diagnosis of PMF (in Patient 1) or MDS (in Patient 2), with undetectable *NPM1* minimal residual disease.

Aline Renneville, M.D., Ph.D.

Centre Hospitalier Régional Universitaire de Lille
Lille, France

Lionel Adès, M.D., Ph.D.

Hôpital Saint Louis
Paris, France

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TO THE EDITOR: Ivey et al. report that the persistence of *NPM1*-mutated transcripts in blood samples after two cycles of chemotherapy was associated with an increase by a factor of 6.8 in the risk of relapse ($P<0.001$) in patients with standard-risk newly diagnosed AML. These findings and others[1,2] suggest a potential role for monitoring measurable residual disease in patients with AML. However, to make these analyses useful to the physician for patient-level decision making, the data should be supplemented by a measure evaluating patient-level prediction, such as a C-statistic. Our experience suggests that even for models that have several covariates with large hazard ratios that are independently significant (at $P<0.01$), C-statistics often range from 0.75 to 0.80.[3,4]

Megan Othus, Ph.D.

Fred Hutchinson Cancer Research Center
Seattle, WA
mothus@fhcrc.org

Elihu Estey, M.D.

University of Washington
Seattle, WA

Robert P. Gale, M.D.

Imperial College London
London, United Kingdom

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THE AUTHORS REPLY: Baccarani asks whether the group of patients with delayed clearance of *NPM1*-mutated transcripts could have been identified on the basis of a suboptimal morphological response to induction therapy. We found that this was not the case. In the combined training and validation data sets involving 437 patients, 88% of patients who tested negative for *NPM1*-mutated transcripts in the peripheral blood and 89% of those who tested positive after two cycles of chemotherapy had a complete remission with blood-count recovery after the first cycle. We found no significant relationship between the response to the first cycle of chemotherapy and status with respect to minimal residual disease after the second cycle; only 2 patients with evidence of minimal residual disease in the peripheral blood after the second cycle had resistant disease after the first cycle, and 6% of patients without minimal residual disease and 4% of those with minimal residual disease had a partial remission after the first cycle. An analysis that was restricted to patients who would be expected to have the best prognosis (i.e., those who were in complete remission after the first cycle of chemotherapy) showed a highly significant difference in survival according to whether *NPM1*-mutated transcripts were undetectable or detectable in peripheral blood after the second cycle of chemotherapy (75% and 24%, respectively; P<0.001). These findings show that the status of minimal residual disease in peripheral blood after the second cycle of chemotherapy adds to the standard response criteria after the first cycle.

Othus and colleagues raise the issue of precision in predicting relapse. As with pretreatment variables,[1] it is not realistic to expect that any minimal-residual-disease test that is applied at a single early time point during therapy will identify exactly which patients are destined to have a relapse after first-line therapy.[2] However, this

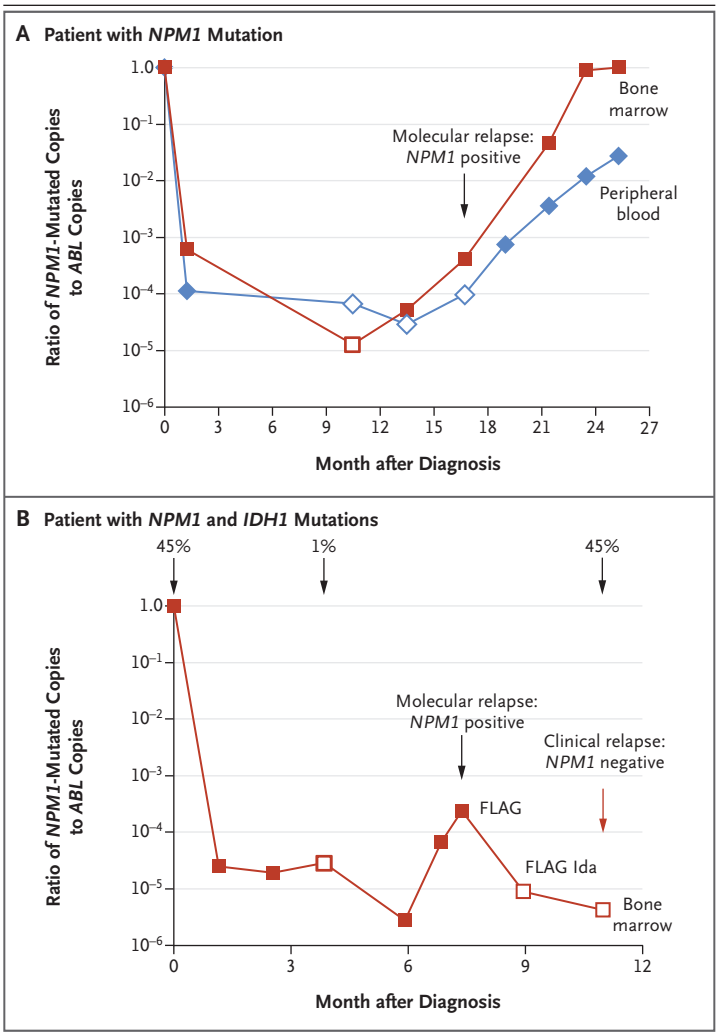

Figure 1. Sequential Monitoring of Minimal Residual Disease and Relapse in AML.

Among 100 patients with *NPM1*-mutated AML who were treated in the NCRI AML17 trial, who were tracked by means of real-time quantitative polymerase-chain-reaction (RT-qPCR) assay, and who were subject to disease recurrence, the *NPM1*-mutated allele was stable at the time of relapse in 98 patients. In these patients, relapse was predicted by RT-qPCR positivity, with a rising level of *NPM1*-mutated transcripts, as exemplified in Patient 17-1792, whose data are shown in Panel A. Solid data points indicate that disease transcripts were detectable, and open data points indicate that they were undetectable. For RT-qPCR–negative samples, data points are plotted according to the maximal sensitivity afforded by the follow-up sample. In 2 of 100 patients, the *NPM1*-mutated allele was not detected at the time of relapse. Data for the first of these patients were presented in our published report, and data for the second patient (number 17-2772) are provided in Panel B. At diagnosis, *NPM1* and *IDH1* mutations were detected. Both mutations persisted in the marrow after first-line chemotherapy. At 7 months after diagnosis, an increase in the *NPM1*-mutated transcript level was detected, which was confirmed in a second sample confirming a diagnosis of molecular relapse. The patient was treated preemptively with salvage chemotherapy, which eliminated the *NPM1*-mutated clone; the patient subsequently had a relapse with *IDH1*-mutated AML. The percentages at the top of the graph indicate the variant allele frequency for *IDH1* R132G. In Panels A and B, the number of *NPM1*-mutated copies has been normalized relative to the number of copies of the Abelson (*ABL*) control gene. FLAG denotes a regimen of fludarabine, high-dose cytarabine, and granulocyte colony-stimulating factor, and Ida idarubicin.

aspiration can be realized in the majority of patients through sequential monitoring of minimal residual disease,[2] as supported by our data (Fig. 1A). We found that the early assessment of minimal residual disease in patients with *NPM1*-mutated AML provides a more powerful predictor of relapse risk than factors that are currently used to inform transplantation decisions[3] in patients with AML. As such, we do not agree that the C-statistic is important in guiding treatment decisions. In our study, a naive application of the C-statistic to the pooled training and validation sets to predict mortality results in a value of 0.65. However, the C-statistic has limitations when applied to a binary marker, as described previously.[4] There is little doubt that a marker that predicts 0% versus 75% survival would be worth knowing about and clinically relevant. Yet if this marker appears in only 10% of the population, the C-statistic is only 0.65. If it appeared in 50% of the population, the C-statistic would be 0.90. And yet the prognosis that is based on the marker is the same in both scenarios.

Morin-Zorman and colleagues describe an interesting series of older adult patients with evidence of preleukemic clones in which the *NPM1*-mutated clone was eliminated by AML therapy but in whom myelodysplasia or myeloproliferative neoplasm subsequently developed. We agree that this phenomenon may be less common in younger patients, who were the focus of our study. Data on older patients are being collected in our ongoing "monitor versus no monitor" randomization on the basis of status regarding minimal residual disease in the NCRI AML17 and AML19 trials. We have now extended our analysis of relapsed *NPM1*-mutated AML to 100 patients (Fig. 1A), which showed the absence of the *NPM1* mutation at relapse in 2 patients. We described the first patient in our published study. The clinical course of the second patient is presented in Figure 1B and serves to highlight the importance of studying multiple time points to fully understand the clonal dynamics underlying relapse.

Robert K. Hills, D.Phil.

Cardiff University School of Medicine
Cardiff, United Kingdom

Adam Ivey, Ph.D.

Guy's Hospital
London, United Kingdom

David Grimwade, Ph.D.

King's College London
London, United Kingdom
david.grimwade@kcl.ac.uk

for the UK National Cancer Research Institute (NCRI) AML Working Group

Since publication of their article, the authors report no further potential conflict of interest.

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